Ubiquitination studied by label-free real-time single molecule optical sensing

This project is one of a number that are in competition for funding from the South West Biosciences Doctoral Training Partnership (SWBio DTP) which is a BBSRC-funded PhD training programme in the biosciences, delivered by a consortium comprising the Universities of Bath, Bristol, Cardiff and Exeter, along with the Rothamsted Research Institute. The partnership has a strong track record in advancing knowledge through high quality research and teaching, in collaboration with industry and government.

Studentships are available for entry in September/October 2019.

All SWBio DTP projects will be supervised by an interdisciplinary team of academic staff and follow a structured 4-year PhD model, combining traditional project-focussed studies with a taught first year which includes directed rotation projects.

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Co-supervisors: Prof Frank Vollmer (University of Exeter), Dr Paul Whitley (University of Bath), Dr Janet Anders (University of Exeter)

Project description

Ubiquitination is an important posttranslational modification involved in modulation and regulation of protein function in many processes in most, if not all, eukaryotic cell types; ubiquitination goes awry in numerous diseases. Ubiquitination involves the covalent attachment to a target protein (the "substrate") of one molecule, or multiple molecules in chains, of a small protein called ubiquitin (Figure 1). As described below, different types of ubiquitin chains can be assembled; this is important because chain type determines the biological outcome of ubiquitination (e.g. whether the substrate protein is degraded, or its function or location is affected), but currently it is difficult to determine chain type. The aim is to develop a rapid, accurate and user-friendly method to identify the type of ubiquitin chain assembled by any E3 ubiquitin ligase, at the same time providing new insights into ubiquitination mechanism and kinetics.

Ubiquitination, catalysed by ubiquitin ligases, involves covalent conjugation of ubiquitin to the protein substrate via formation of an isopeptide bond between ubiquitin's C-terminal carboxylate and the substrate's N-terminal amine or epsilon-amino group of a lysine residue. In many cases a chain of ubiquitin molecules is assembled on a substrate protein (Figure 1) whereby a ubiquitin monomer is linked to the chain via any one of seven lysines or N-terminus. Ubiquitin chains can involve a single type of lysine linkage or mixed linkages, with each linkage producing a different degree of flexibility and repertoire of conformational states. Since chain type determines biological outcome, this project will provide detailed new insights into the relationships between E3 ligase function and resulting phenotype, and will therefore potentially advance understanding of the relationships between particular ubiquitin ligases and health and disease.

The project will involve a combination of cutting edge physical and biochemical methods. Ubiquitination reactions will be studied using plasmonically enhanced whispering gallery mode (WGM) microcavity sensing (Figure 1). This is the first optical technique capable of directly monitoring structural changes within individual biomolecules such as proteins. A major aim will be establishing whether each type of ubiquitin linkage has a unique WGM signature. Biochemical and chemical

biology experiments with Drs Bagby and Whitley at Bath will include ubiquitination assays, and production of ubiquitin and enzymes (ligases and deubiquitinases) modified for immobilisation on gold nanoparticles that are used in WGM sensing. Single molecule WGM sensing studies of ubiquitin chain assembly will be conducted at Exeter with Professor Vollmer's group.

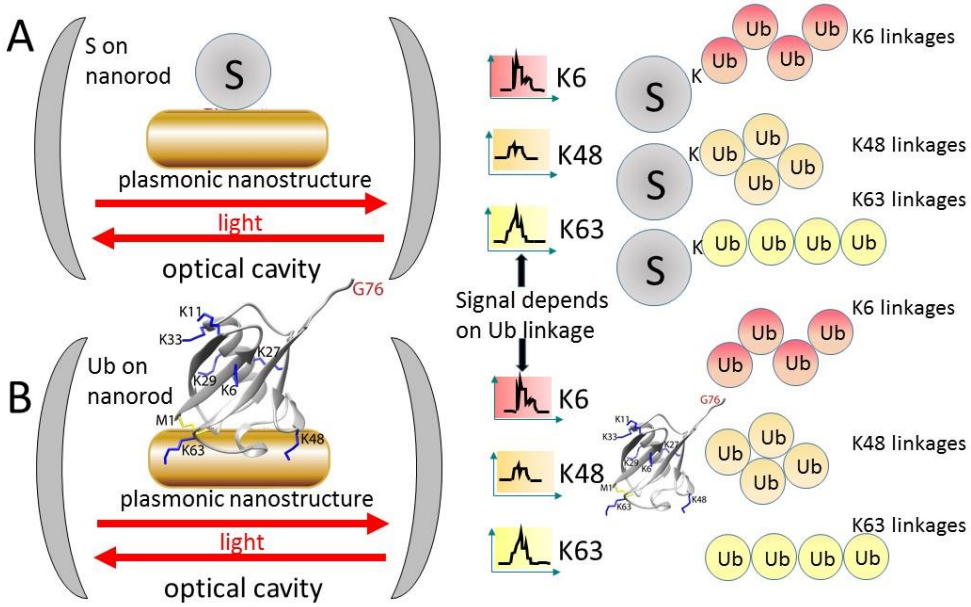

Figure 1 Overall concept. Signal upon ubiquitin (Ub) conjugation to (A) substrate (S) or (B) Ub immobilised on a gold plasmonic nanorod should vary depending on which Lys of Ub forms the new linkage (K6-, K48-, K63-linked Ub chains as examples).

Funding

Studentships provide funding for a stipend at the standard UKRI rate (currently £14,777 per annum, 2018/19 rate), research and training costs and UK/EU tuition fees for 4 years.

UK and EU applicants who have been residing in the UK since September 2016 will be eligible for a full award; a limited number of studentships may be available to EU applicants who do not meet the residency requirement. Applicants who are classed as Overseas for tuition fee purposes are not eligible for funding.

Applications

Applicants must have obtained, or be about to obtain, a First or Upper Second Class UK Honours degree, or the equivalent qualifications gained outside the UK, in an appropriate area of science or technology.

Applications should be submitted on the University of Bath's online application form for a PhD in Biosciences. Please ensure that you quote the supervisor's name and project title in the 'Your research interests' section. You may apply for more than one project if you wish but you should submit a separate personal statement relevant to each one.

The deadline for the receipt of applications is Monday 3 December 2018.